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## **PACKING BLACK RIPE OLIVES IN ACID CONDITIONS**

Concepción Romero, Manuel Brenes, Pedro García-Serrano, Alfredo Montaña,  
Eduardo Medina, Pedro García-García\*

*Food Biotechnology Dept., Instituto de la Grasa (IG-CSIC)*

*Ctra. Utrera km 1, Building 46, 41013 Seville, Spain.*

Corresponding author: P. García-García (E-mail: [pedrog@cica.es](mailto:pedrog@cica.es)).

## **Abstract**

The type of container (airtight and pouches with different O<sub>2</sub> permeability) and packing conditions (cover brine, air or N<sub>2</sub> atmosphere) has been studied to preserve black ripe olives in acid medium for a year. Unlike the traditional sterilized product, these acidified olives only needed pasteurization to assure its microbial safety, the absence of acrylamide being an additional advantage. Surprisingly, an increase in the oxygen diffusion through the films (i) faded the black color of the olives, (ii) softened the fruit that lost around 33 % of its initial firmness in only 6 months, and (iii) produced the lipid's oxidation forming volatile compounds that transmitted an abnormal flavor which tasters identified as rancid. Therefore, ripe olives in acid medium must be packed in airtight containers such as glass jars, cans or metallic pouches with cover brine or N<sub>2</sub> atmosphere. The addition of calcium is recommended to avoid olive softening.

**Key words:** Acid; Black ripe olive; Lipid oxidation; Oxygen; Packing

### ***Highlights***

- Ripe black olives in acidic medium are free of acrylamide.
- They must be packed in airtight containers with cover brine or N<sub>2</sub> atmosphere.
- Minimal presence of O<sub>2</sub> oxidizes the lipid and transmits rancid flavor to the olives.
- They faded and softened slightly during shelf life. Ca improves the firmness.

## 1. Introduction

Olives may be processed to produce three main table products known as Spanish-style green olives, naturally black olives, and black ripe olives. The latter are also known as Californian-style and the elaboration method involves a first stage of fruit preservation in an acidic solution (De Castro, García, Romero, Brenes & Garrido, 2007; García-Serrano, Romero, Medina, García-García, De Castro & Brenes, 2020), followed by a darkening stage that nowadays consists of treating the olives 1-2 times with a dilute NaOH solution (lye) followed by tap water washes (Romero, García-García, & Brenes, 2016; Brenes, Romero & García-García, 2017). Subsequently fruits are immersed in a ferrous solution (lactate or gluconate) for 6-24 hours, and during all the process air is bubbled through them (Sánchez-Gómez, García-García, & Rejano-Navarro, 2006). According to the pH of the final product (6.0-7.0), black ripe olives can be considered a “low-acid canned food” hence this product must be canned in an airtight container and sterilized to ensure microbial safety.

If prior to packaging or during this stage, acid is added to reach a pH at equilibrium lower than 4.6 the product would be qualified as “acidified food” much like dill pickles, hot sauce, and pickled fish (FDA, 2020a). Consequently, acidified black ripe olives would require pasteurization only to ensure safety. This change could be advantageous since it would save energy, reduce investment in retorts and reduce labor. In addition, the elimination of the sterilization stage would be very positive for the reduction of acrylamide in this product.

Acrylamide has been identified as a probable carcinogen (National Toxicology Program, 2019) and it is found in thermally treated plant-derived foods (>100-110 °C) as cereals, potatoes, coffees and ripe olives (Friedman, 2003; Zyzanski et al., 2003; Casado & Montaña, 2008) but it is not detected in unheated foods (<100-110 °C) (Tareke, Rydberg, Karlsson, Eriksson & Törnqvist, 2002).

Acrylamide has been found in black ripe olives in a wide range (225-1925 µg/kg) due to the sterilization process at temperatures above 110 °C (FDA 2020b; Casado & Montaña, 2008; Charoenprasert & Mitchel, 2014). Despite many studies on this issue, the optimization of the heat treatment is nowadays the only industrial practice to reduce the level of this compound ensuring safety (Tang et al., 2016). In contrast, Spanish-style green and naturally black olives are pasteurized at a temperature below 90 °C due to their pH being below 4.6 units, where the acrylamide content is negligible (Charoenprasert & Mitchel, 2014). Therefore, the acrylamide content in acidified black ripe olives would be negligible because a pasteurization treatment would only be necessary to ensure safety during their shelf life.

Conversely, there is an increasing demand for table olive products without cover brine because consumers prefer seeing the olives without any black liquid surrounding them, and because getting rid of the liquid once the containers are opened is also a problem. In addition, management of black ripe olives without cover brine would mean transporting half of the normal container weight. Therefore, pouches are an alternative to be thoroughly considered in deep for the packing of black ripe olives in acidic conditions.

As is well-known, the permeability of oxygen through the films has a great influence on the color, and on most of the organoleptic characteristics of the packed food. In the case of table olives, it has been reported that the diffusion of oxygen inside the pouches may cause darkening on Spanish-style green olives (Sánchez, López-López, Beato, De Castro & Montaña, 2017a), the higher the oxygen permeability of the film, the darker the olives will become (Sánchez, De Castro, López-López, Cortés-Delgado, & Montaña, 2017b). Similar results have been found for green olives packed in plastic pouches during the pasteurization step (Piscopo, De Bruno, Zappia & Poiana, 2016). Thus, a darker color would be expected in acidified olives packed in pouches with high oxygen permeability. However, it was not the case for black ripe olives packed in pouches at the current neutral pH (Romero, Brenes, García-Serrano, Medina, García-García, 2019).

It must be noted that black ripe olives discolor as the pH decreases below 6-7 units (Brenes, Romero, García, & Garrido, 1995) yet it has also been found that the diffusion of oxygen inside pouches gives rise to darker olives packed with cover brine in acid conditions (García, Brenes, Romero, & Garrido, 1999).

Moreover, it has been reported that the flavor of black ripe olives packed in tins or jars can be preserved for 3 years (García-García, Sánchez-Gómez & Garrido-Fernández, 2014). However this information is not available for black ripe olives packed in pouches under acidic conditions. It must be noticed that table olives contain a high amount of oil, prone to be oxidized by the oxygen diffused into the pouches which could transmit a rancid sensation to the product

(Kanavouras, Hernández-Muñoz & Couteliers, 2006; Zhu, Wang & Shoemaker, 2014).

The aim of the present work was to investigate the packing of black ripe olives under acidic conditions (i) in different type of containers (airtight container or plastic pouches with different oxygen permeability), and (ii) different packing conditions (liquid, air or controlled atmosphere), in order to mimic the characteristics of the traditional product.

## **2. Material and methods**

### *2.1 Olives*

Fruit of the Hojiblanca cultivar (*Olea europaea* L.) stored for a period of 15 months in fermenters holding 22 kg of olives at Instituto de la Grasa pilot plant in acidic conditions were used for the assays (García-Serrano et al., 2020).

### *2.2. Darkening process*

Olives (6 kg) were placed in four cylindrical oxidation chambers as explained in detail elsewhere (Brenes et al. 2017), and immersed in 6 L of a NaOH solution (30 g/L) until it penetrated to the stone. Subsequently, the lye was removed and the olives covered with tap water/previous preservation liquid (1/1) for 20 h. The fruits were then put in new tap water for another day and the

pH of the liquid was maintained at 8.0 units by the bubbling CO<sub>2</sub>. Finally on the third day the fruits were covered with a ferrous gluconate solution (1 g/L) for 5 h to fix the black color formed. Air was bubbled (200 L/h) into the oxidation chambers throughout the whole process.

### *2.3. Acidification and packing*

Darkened olives were pitted with an industrial machine (OFM, Dos Hermanas, Spain) and 4.3 kg of the fruit were put in each of the four oxidation chambers. Subsequently, they were covered with 5.2 L of brine containing 50 g NaCl/L, 0.3 g ferrous gluconate/L to prevent discoloration, and 9.2 mL lactic acid/L (Panreac, Spain) to adjust the pH of the packed olives at around 4.0-4.2 units at equilibrium. Additionally, CaCl<sub>2</sub> was added in one of the tanks to reach a concentration of 1600 mg/L of calcium concentration.

After 20 hours of bubbling air into the tanks, olives were packed in 314 mL volume glass jars (JUVASA, Dos Hermanas, Spain) and three types of plastic complex pouches (SPgroup, Villarrubia, Spain): (i) Polyester and white polyethylene (PET+PE B MD) with an O<sub>2</sub> permeability < 96 cm<sup>3</sup>/m<sup>2</sup>/day; (ii) Biaxially oriented polyamide and polyethylene (OPA+PE MD) with an O<sub>2</sub> permeability < 30 cm<sup>3</sup>/m<sup>2</sup>/day), and (iii) High barrier polyester and polyethylene (PET PVDC PAST+PE MD) with an O<sub>2</sub> permeability <10.5 cm<sup>3</sup>/ m<sup>2</sup>/day. The technical characteristics of the films are shown in Table S1.

An amount of 145 or 100 g of pitted olives with 175 and 120 mL of cover brine were introduced into the glass jars and plastic pouches respectively.



When cover brine was not used, the internal atmosphere of the jars and pouches was 175 and 40 mL respectively. The inner atmosphere of the jars was air, and in some cases, it was modified in the pouches with nitrogen, which were then sealed with a Tecnotrip sealer mod EVT-7-G-TD-SD (Terrasa, Spain).

#### *2.4. Pasteurization and storage*

Glass jars and pouches were pasteurized at 80 °C in a computer-controlled Steriflow retort (Madinex, Barcelona, Spain) for the time needed to achieve the same lethality value, 15 UP<sub>62.4</sub> (pasteurization units at 62.4 °C) (IOC, 2004). Temperature inside the jars and pouches was controlled with cylindrical (17 mm Ø and 5 mm height) programmable thermometers (Thermotrack PC, Progres-Plus, France). It must be noted that these Thermo buttons were of a similar size than olives.

Finally, all containers were stored at ambient temperature at the Instituto de la Grasa pilot plant (15-30 °C) for one year.

#### *2.4. Design of assays*

Olives (with only pH correction and also with Ca addition) were packed in airtight containers (glass jars) and plastic pouches with (i) in the same brine in

which they had remained for 20 h after pitting, (ii) in air atmosphere, and (iii) in N<sub>2</sub> atmosphere.

Two containers of each type of olive treatment were analyzed the day after packaging (0 time) and at 2, 6 and 12 months for chemical, superficial color and firmness parameters of olives. Also, at one year, a sensory analysis was performed and the volatile compounds in the fruits were analyzed.

## *2.5. Microbiological and physico-chemical analyses*

The absence of microbial growth in the packings was achieved by mixing 36 g of fruit with 25 mL of sterile saline solution (0.9 % NaCl) in a stomacher bag. Pulp was homogenized for 1 min at maximum speed (300 rpm) in the stomacher Seward 400 (Seward Medical Ltd., West Sussex, UK). One mL of the suspension was plated in PCA medium (Oxoid, Basingstoke, Hampshire, UK) in triplicate and incubated at  $30 \pm 2$  °C for 6 days.

The concentration of NaCl, pH and free acidity in the cover brine were measured according to traditional methods for table olives (Brenes et al., 2017). The pH of the olives was measured by puncturing the flesh of 10 fruits with a pH Spear instrument (Eutech Instruments, Thermo Scientific).

The variation in the weight of the olives during their shelf life was measured by weighing the drained fruits before packing, and at the time of analysis.

The surface color of the fruits was measured by using a BYK-Gadner Model 9000 Color view spectrophotometer (Silver Spring, MD, USA) and it was

expressed as reflectance value at 700 nm ( $R_{700}$ ). Lower reflectance values indicate darker fruit. Any interference from stray light was minimized by covering the samples with a box, which had a matte black interior. Results were the mean of 10 determinations.

Firmness, measured as the shear compression force in Newtons (N) to break 3 pitted olives, was determined using a Kramer shear compression cell coupled with TA.TXplus Texture Analyser (Stable Micro System, Surrey, UK). The cross-head speed was 200 mm/min. The indicated value was the mean of 8 measurements.

Concentrations of O<sub>2</sub> and CO<sub>2</sub> in the inner atmosphere of containers were measured with a Gaspac Advance Headspace/MAP Analyzer (Illinois Instruments, Inc. Johnsburg, IL, USA) by introducing a hypodermic needle into plastic pouches or a steel piercing head into the metallic closure of glass jars.

Dissolved O<sub>2</sub> concentration in the cover brine was analyzed by introducing the probe HI98193 Dissolved Oxygen (Hanna Instruments, Eibar, Spain) in a beaker with about 30 mL of cover brine, taking care that it did not aerate in the transfer from the container.

The Ca content in olive flesh and in surrounding liquids was analyzed by wet digestion and atomic absorption, following the method described by García-Serrano et al. (2020).

Volatile compounds were analyzed by headspace solid-phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS), following the method described by Sánchez et al. (2018) with few modifications, i.e. 5-nonanol as internal standard instead of 3-

octanol, 40 °C as extraction temperature instead 60 °C, and use of a 2 cm long SPME fiber (Supelco, Bellefonte, PA) instead of 1 cm. Identification was carried out by matching compounds mass spectra and retention indexes with those of authentic standards in NIST 17 library and retention index databases.

Acrylamide was analyzed according to the method described by Casado & Montaña (2008), in which the analyte was detected as 2-bromopropenamide by GC-MS, employing ( $^{13}\text{C}_3$ ) acrylamide as internal standard.

## *2.6. Sensory analyses*

The acidified ripe olives were tested according to the "Method for sensory analysis of table olives" in the Instituto de la Grasa's normalized testing room (IOC, 2011). This method classifies olives commercially through the use of descriptors related to the perception of negative sensations ("abnormal flavor"). Gustatory attributes (salty, bitter, and acidic) and kinesthetic sensations (hardness, fibrousness, and crunchiness) were also assessed.

Three or four olives were presented to each of the eight trained tasters in a normalized glass (Glass for oil tasting, COI/ T.20/Doc). Panelists had to indicate the intensity they perceived for each of the attributes in a 10 cm scale. The left extreme denoting the absence of an attribute while the right end the maximum perception. The intensities of the attributes ranged from 1 to 11 and were measured using a ruler.

The values of the attributes were expressed as the median of the individual data and the variability by robust standard deviation.

## *2.7. Statistical analyses*

Statistica software 7.0 was used for data processing (Statistical for Windows, Tulsa, OK, USA). Comparison between means was made with Duncan's multiple range test and the differences were considered significant when  $p < 0.05$ , except in the sensory analysis where differences were considered significant when confidence intervals of the medians ( $p < 0.05$ ) did not overlap.

Principal component analysis (PCA) based on the content of selected volatile compounds was performed with SIMCA 14.1 software (Umetrics, Umea, Sweden).

## **3. Results and discussion**

### *3.1. Pasteurization assays and safety*

Specific heat treatments were developed for each container (glass jar or pouch) and packaging conditions (with cover brine and without liquid) to reach an accumulated lethality of 15 units ( $15 UP_{62.4}$ ). The lack of cover brine delayed heat penetration inside both jars and pouches (Figures S1, A and B), as has also been detected for the sterilization of traditional black ripe olives (Romero et al., 2019). Consequently, the duration of the heat treatment was extended in the containers without liquid so that all the packages had the same accumulated lethality units (Figures S1, C and D). Logically, the time needed to pasteurize

olives in pouches was always lower than in jars, given the better heat transmission through the plastic film.

According to the data shown in Figure S1, maintenance time at 80 °C was 4 min for glass jars, and 2 min for pouches both with cover brine. In packaging without liquid, 24 min and 11 min were needed to achieve the same accumulated lethality (15-18  $UP_{62.4}$ ) for glass jars and pouches respectively.

The accumulated lethality of 15 units (15  $UP_{62.4}$ ) was initially established for the pasteurization of Spanish-style green olives and it has also been applied for heat treatment of natural black olives (IOC, 2004). However, black ripe olives packed under acid conditions would be a new product, so it would be necessary to check the efficacy of the standard pasteurization. Consequently, microbial analyses of the olive pulp was performed at 12 months of storage with negative results in all cases. In addition, the pH of liquids and olives decreased slightly over time, 0.1-0.2 units after one year. A similar decrease has also been observed in commercial black ripe olives packaged in neutral conditions during their shelf life (García-García, Sánchez-Gómez & Garrido-Fernández, 2014).

### *3.2. Evolution of $O_2$ and $CO_2$*

The initial concentration of oxygen (21 %) decreased rapidly in all type of containers (glass jar and pouch) during the pasteurization stage of olives packed under air atmosphere (Figure 1), and the consumption of oxygen continued to a large extend for the first two months after packing regardless of

the type of container, which is a trend also observed for traditional black ripe olives packed without cover brine (Romero et al., 2019).

In the case of airtight containers (glass jars), the O<sub>2</sub> concentration remained constant in the internal atmosphere after the second month of storage, O<sub>2</sub> consumption stopped; obviously there was no gas exchange between the glass jar and the external atmosphere due to the tightness of the container. In contrast, oxygen almost disappeared in pouches with less permeability after one year, while increasing with time as the permeability of the pouch was higher (Figure 1).

Furthermore, the concentration of oxygen in the inner atmosphere of pouches packed under nitrogen increased with time, and it was remarkable for films with oxygen permeability higher than 30 cm<sup>3</sup>/m<sup>2</sup>/day.

With regard to CO<sub>2</sub> concentration in the inner atmosphere of the glass jars and pouches, an increase of this gas was detected after pasteurization in all types of containers, a trend continued in jars and pouches with the lowest permeability for 2 months, thereafter observing a drop of the concentration of CO<sub>2</sub> with time was observed (Figure S2). The decline of CO<sub>2</sub> in the pouches with higher oxygen permeability started even after pasteurization, which may also be related to the high permeability of this gas through these films (Table S1). This drop of CO<sub>2</sub> concentration with time was also detected in pouches packed under nitrogen atmosphere with oxygen permeability <30-96 cm<sup>3</sup>/m<sup>2</sup>/day; while a steady increase with time occurred with the oxygen permeability film of <10.5 cm<sup>3</sup>/m<sup>2</sup>/day (Figure S2).

There is no explanation for the consumption of  $O_2$  and the production of  $CO_2$  because, as has been mentioned before, there was no growth of microorganisms in these containers, and respiration of olives ends after the first month of processing (Romero, Brenes, García & Garrido 1996). This also took place in the packaging of ripe olives in neutral conditions (Romero et al., 2019).

When black ripe olives were packed with cover brine, there was a decrease in the initially dissolved  $O_2$  from  $\approx 6.5$  mg/L to values below 0.5 mg/L in most containers (Figure 2). As mentioned above oxygen consumption was also observed in olives packed with cover brine for packaging without liquid. In addition, the level of oxygen in the brine of olives packed in jars did not change with time; while it increased with time in pouches, the rate being higher at higher film permeability.

### *3.3. Superficial color and firmness of the product*

The main quality characteristic of black ripe olives is precisely their shiny black color which is very appreciated in most international markets. However, this black color depends on the pH of the olives, the lower pH the less dark the olives are (Brenes et al., 1995; García et al., 1999). Hence, the acidification of the olives before packaging and pasteurization led to less dark olives than those obtained after the darkening stage. However, they still retained a very good black color with  $R_{700}$  values around 6.3-6.5 units. Nonetheless, the storage of the acidified ripe olives at ambient temperature caused an increase of the reflectance with time, particularly in olives packed under air atmosphere without



cover brine and also in olives packed with cover brine (Figure 3), which happened despite the type of container (glass jar or pouch). It has been reported that traditional black ripe olives fade during their shelf life, associated with a decrease of the olive pH with time (García-García et al., 2014); yet this is not the case with acidified black ripe olives as they maintained their pH around 4.0-4.2 units after one year.

In contrast, olives packed under nitrogen atmosphere did not fade with the one year of storage time, irrespective of the oxygen permeability of the film (Figure 3). Hence, this is the best packing method to preserve the black color of the acidified product.

Regarding the firmness of olives, pasteurization led to a slight decrease of this parameter from 303 N to 270-290 N to break 3 pitted olives, which was a loss of texture, much lower than reported when traditional black ripe olives were subjected to the mandatory sterilization stage at a temperature close to 121 °C (Romero, García, Brenes & Garrido, 1995).

As has been shown in Figure 4, the firmness of the olives decreased in all cases with time, although the rate of softening was faster in pouches with high oxygen permeability, regardless of the inner atmosphere of the containers or whether the olives were packed with cover brine. Indeed, García et al. (1999) found differences in firmness of acidified black ripe olives that were packed with cover brine prompted by the type of pouch, and they indicated that the oxygen-permeable plastic bags gave rise to softer olives than those preserved in aluminum pouches.

It is worth noting that the softening rate of vegetables including olives increases as the pH decreases from neutral to acidic values (McFeeters, Brenes, & Fleming, 1995; Brenes, García & Garrido, 1994). Consequently, firmness of traditional black ripe olives diminished 20-30 % after storage at ambient temperature for 3 years (García-García et al., 2014). While acidified black ripe olives reduced their texture more than 33 % in just 6 months (Figure 4). Taking into consideration this softening effect at acid conditions, in a parallel assay the acidic medium was spiked with calcium chloride before packing, and it increased the content of calcium in the olives from 688 mg/kg to 1580 g/kg thereby softening rate of the olives was partially inhibited regardless of the type of container and inner atmosphere of the pouches (Figure 4). Therefore, the highest texture was obtained in olives previously immersed in the calcium chloride solution and packed under nitrogen atmosphere.

#### *3.4. Weight loss and acrylamide content*

Olives packed with cover brine lost only around 1 % of their weight after one year of storage at ambient temperature, despite the type of container used (glass jar or pouches with different oxygen permeability). In contrast, packing olives without cover brine gave rise to a steady loss of weight with time (Figure 3S), this loss being higher than 7 % after one year in the case of the product packed in pouches. The glass jar is airtight and does not allow exchanges with the outside, while the pouches are permeable to water vapor, so the loss of weight must be due to the loss of moisture through the film. No differences were

found between the different types of pouches because water vapor permeability was similar in the three types studied (Table S1).

As expected, the analyses carried out on olives packed in each of the types of packaging (glass jar and pouches) showed absence of acrylamide. Hence, the pasteurization treatment of acidified black ripe olives was not energetic enough to produce acrylamide in contrast to what happens with the traditional product at neutral pH which must be sterilized (FDA 2020b, Casado & Montaña, 2008; Charoenprasert & Mitchel, 2014). According to this data, acrylamide was not found in pasteurized Spanish-style green and natural black olives (Charoenprasert & Mitchel, 2014).

### *3.5. Sensorial evaluation and volatile compounds*

Representative containers of the experiences were chosen to carry out a sensory evaluation according to the ICO method (2011). As can be seen in Table 1, panelists did not find differences in the perception of two gustatory sensations (salty and bitter) and two kinaesthetic (fibrousness and crunchiness), the median values being similar to those obtained for commercial samples of black ripe olives (García-García et al., 2014). However, the median of the acid sensation (4.0-5.0) was higher than in the traditional product (1.7-2.5) due to the lactic acid addition performed to lower the pH.

With respect to the hardness sensation, there were differences between the different types of packaging, so the panellists rated as harder those olives which

were packed with cover brine in the airtight container (glass jar) and in the bag with less oxygen permeability, which is in agreement with data depicted in Figure 4.

On the other hand, the work team did not detect off-flavors when the containers were opened at 2 and 6 months but a strong musty odor was perceived in many of the glass jars and pouches after one year of storage, especially in those packaged with air atmosphere.

As can be seen in Table 1, olives packed in glass jars with cover brines were the only ones with the perceived defect of "negative sensations" (abnormal flavor) which did not exceeded the 3.0 value threshold; that this product could be considered as an "extra" commercial category. Likewise, panelists also detected the presence of abnormal flavor in olives packed with cover brine in pouches with the lowest oxygen permeability ( $<10.5 \text{ cm}^3/\text{m}^2/\text{day}$ ), which they identified as rancid, with a defect value of 3.5, which qualifies for the "first" category. Surprisingly, higher values ( $>5.0$ ) were obtained for olives packed in pouched even under nitrogen atmosphere. Definitely, the presence of oxygen during the storage of acidified black ripe olives leads to the development of off-flavors formed with more intensity in olives packed without cover brine and films with increasing oxygen permeability.

At the same time, the volatile compounds determination was carried out in these sensory analyzed olives, mainly considering the aldehydes, ketones and alcohols, which are frequent indicators of lipid oxidation (Franquel, 1983).

The predominant volatiles in the olives packed in pouches were the aldehydes hexanal, octanal, (*E*)-2-heptenal, (*E*)-2-octenal, nonanal, and (*E*)-2-

decenal (Table 1). Hexanal, (*E*)-2-heptenal, and (*E*)-2-octenal could be formed by the breakdown of linoleato hydroperoxides; and octanal, nonanal, and (*E*)-2-decenal by the decomposition of oleato hydroperoxides (Frankel, 1983; Kanavouras et al., 2006; Neugebauer, Granvogl & Schieberle, 2020). These compounds, among other volatile aldehydes, are mainly responsible for potent off-flavors found in virgin olive oil (Zhuet al., 2014). Thus, (*E*)-2-decenal was found to be the most relevant compound in rancid olive oil. In addition to the above mentioned aldehydes, in the olives packed in pouches we must highlight presence of 1-octen-3-ol, which was not detected in the olives packed in glass jar. Garrido-Delgado, Dobao-Prieto, Arce & Valcárcel (2015) identified 1-octen-3-ol along with octanal, ethyl butanoate, and 2-heptenal as the characteristic compounds of “lampante” olive oils with known defects (mustiness-humidity, fustiness and rancidity).

In accordance with the above mentioned, the formation of these volatile compounds must be related to the presence of oxygen inside the containers. Thus, in the hermetic container (glass jar), the O<sub>2</sub> dissolved in the cover brine was initially consumed (Figure 3), and the lowest production of volatiles was observed; additionally, the panelists found no abnormal flavor (Table 1).

In the case of pouches, the lowest volatile formation in olives was found when they were covered with brine and the film had the lowest oxygen permeability (<10.5 cm<sup>3</sup>/m<sup>2</sup>/day); where a limited amount of O<sub>2</sub> penetrated (Figure 2) and was sufficient to generate a greater concentration of volatiles from lipid oxidation, in comparison with the ripe olives in glass jars (J-B-0) (Jar-Brine-0 permeability). As a consequence, the panelists found significant

differences regarding abnormal flavor between the samples P-B-L (Pouch-Brine-Low permeability) and J-B-0 (Table 1).

In the same type of pouch but with N<sub>2</sub> as inner atmosphere (P-N-L, Pouch-N<sub>2</sub>-Low permeability) some O<sub>2</sub> penetration was observed (Figure 1), which prompted that provoked a greater extent of lipid oxidation producing a greater concentration of volatiles compared to pouch with cover brine (P-B-L). This resulted in a higher score for the abnormal flavor sensation in sample P-N-L in comparison with P-B-L (Table 1).

A greater amount of volatile compounds from lipid oxidation were found in the pouch with the highest oxygen permeability (P-B-H) in comparison with the lower (P-B-L) because oxygen penetrated more easily in the former, thus increasing the concentration of dissolved oxygen (Figure 2). It appears that dissolved oxygen serves as a starter for oxidation, as is the case in bulk virgin olive oil (Johnson & Decker, 2015).

As expected, the highest production of volatile compounds related to the rancid sensation and the worst score by the panelists, occurred when olives were packaged in air atmosphere (P-A-L). Moreover, there was a good correlation ( $R^2=0.96$ ) between the total concentration of volatile compounds derived from lipid oxidation and the median values of the abnormal flavor found by the panel (Figure S4).

SPME-GC/MS data were subjected to PCA (Figure 5), and two components accounted for 93% of the variation in the dataset, PC1 explained 70.3% of the variance, whereas PC2 accounted for an additional 22.7%. It is important to point out that the higher the distance between two parameters, the

lower their correlation. In the biplot, all five samples were distinctly distributed and a clear separation achieved. All volatile compounds were located on the right part of the plot.

The samples J-B-0 and P-B-L appeared to be similar and lay on the negative PC1, presenting a different pattern from the other samples. The sample that was most highly related to oxidation-derived volatiles was P-A-L, followed by P-B-H. The sample P-A-L, which had the highest positive PC1 score, was mainly correlated with (*E*)-2-decenal, which presented the highest content for this sample. Whereas P-B-H was particularly related to 1-octanol, pentanal and heptanal, which presented the highest content for this sample (Table 1). The sample P-N-L was located close to zero and was not clearly associated with any volatile compound.

#### **4. Conclusions**

Black ripe olives were packed as acidified food ( $\text{pH} < 4.6$ ) and the pasteurization treatment was intense enough to ensure their microbial preservation during shelf life. In addition, this product was free of acrylamide as opposed to the traditional product at neutral pH that requires sterilization.

Although the packing of acidified black ripe olives under nitrogen atmosphere gave rise to a product with good color and texture, all the types of pouches tested permitted the diffusion of oxygen inside the container, which led to the formation of volatile compounds that transmitted an abnormal flavor,

identified as rancid by testers. Logically, the oxidation was more intense as the permeability of the film was higher and under air atmosphere.

Overall, these findings suggest that black ripe olives in acidic medium must be packed with brine in an airtight container, as shown in this paper with the use of glass jars, although they could also be metallic pouches. It would be possible to use a N<sub>2</sub> atmosphere in an airtight container, and the results could be very similar to that of packaging with cover brine in a glass jar. In both cases, the addition of Ca prior to packaging would improve the firmness of black ripe olives in acidic medium.

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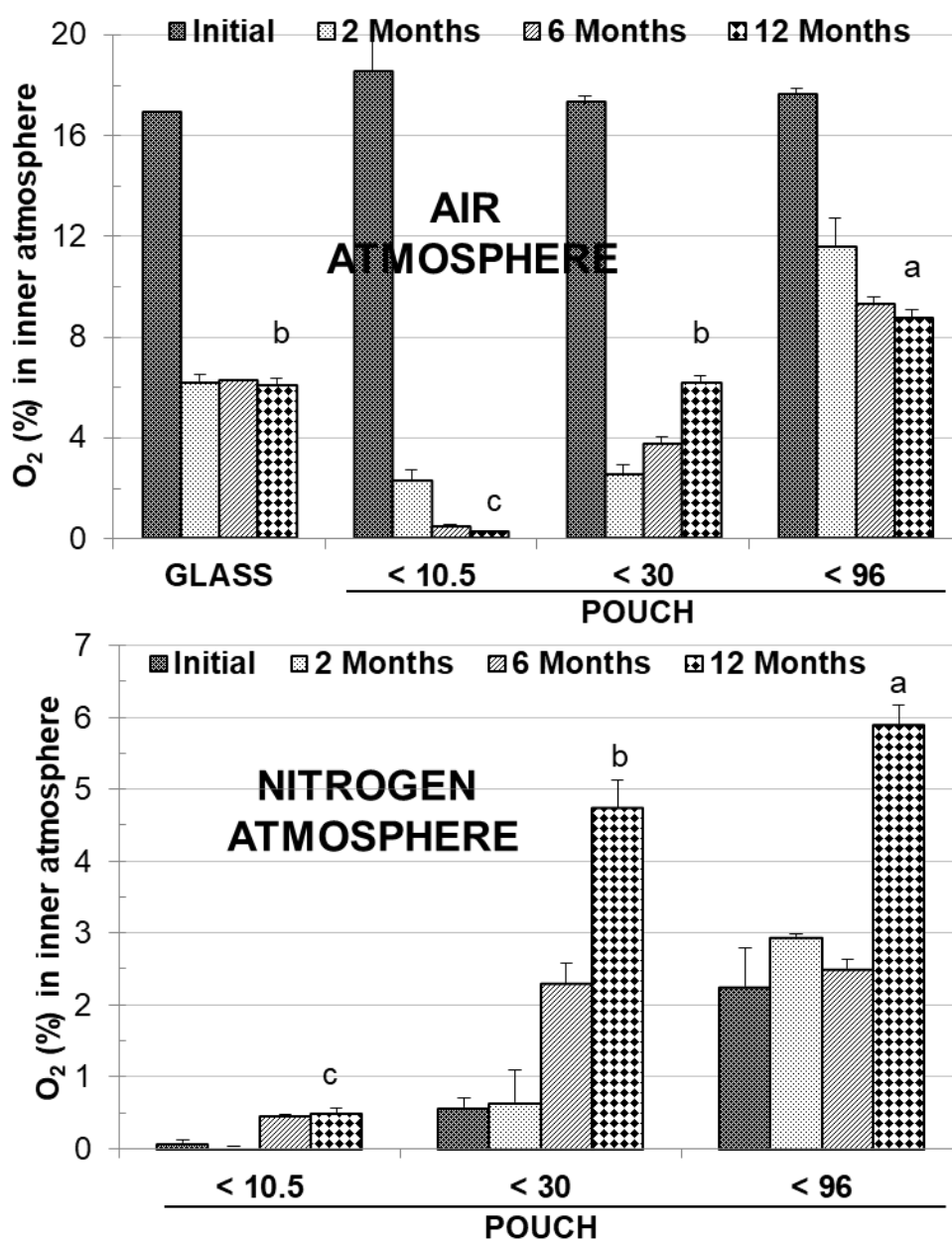
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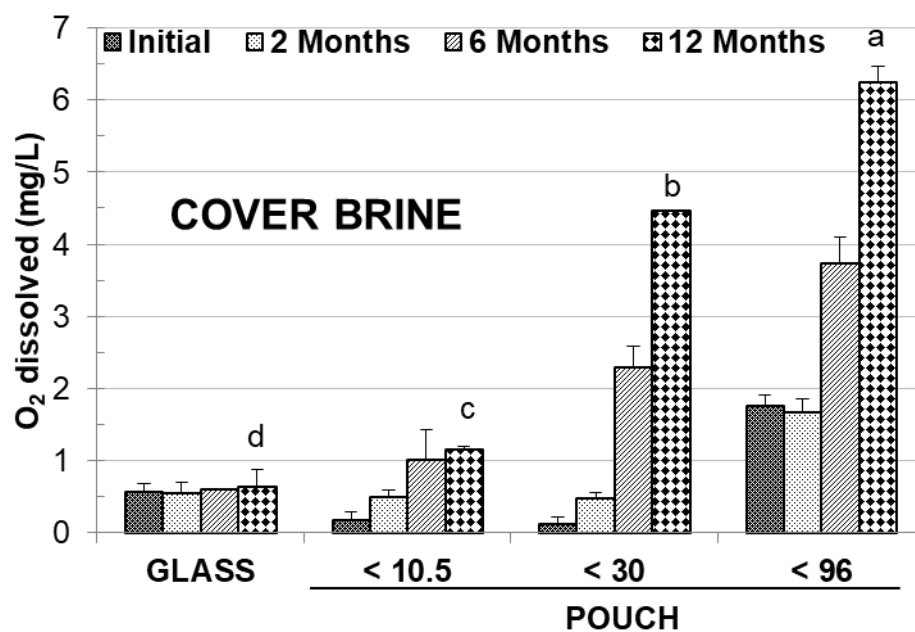
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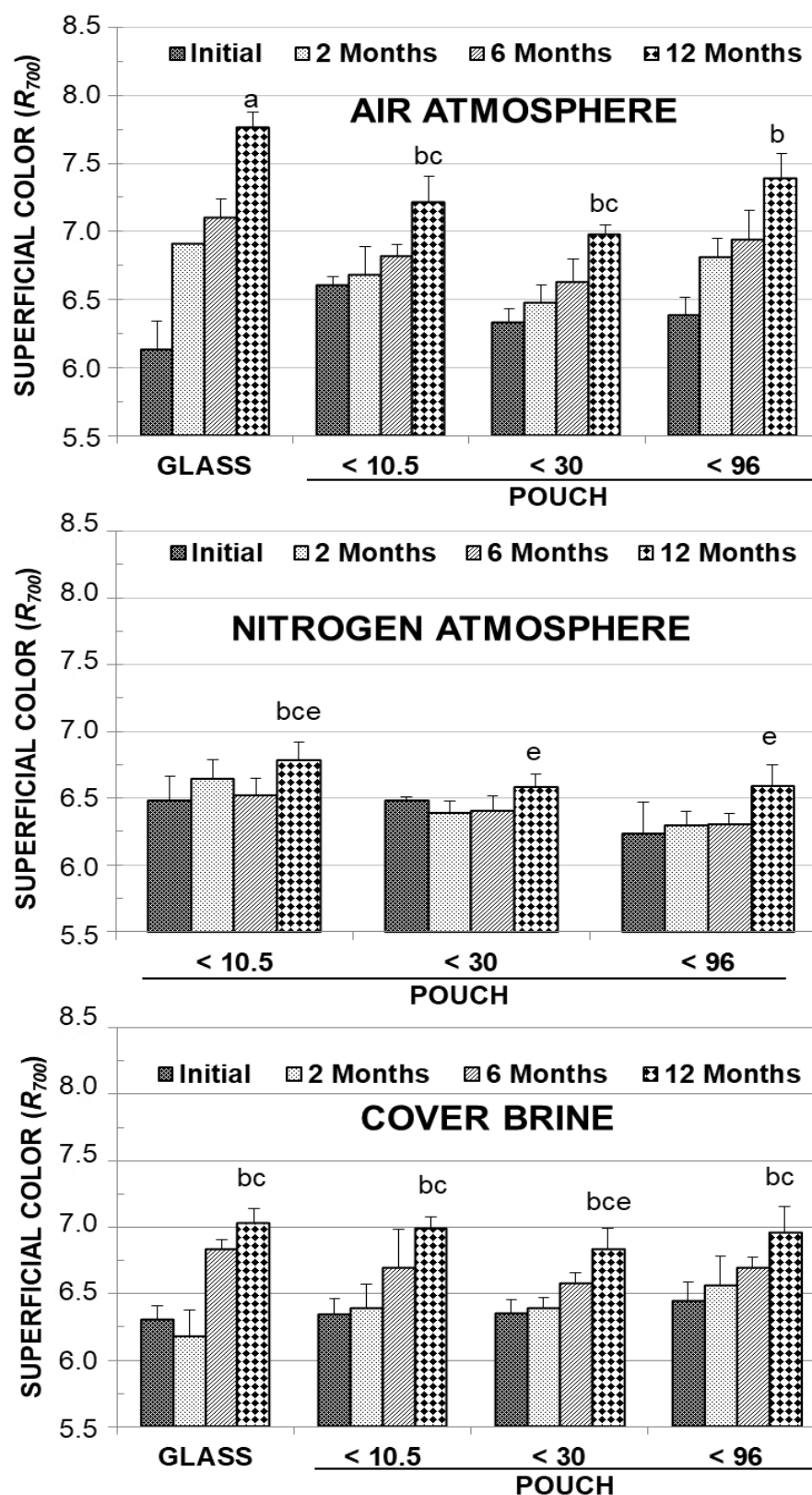


**Figure 1.** Evolution of  $O_2$  (%) in the inner atmosphere of jars and pouches containing acidified black ripe olives packed with air or nitrogen atmosphere. Vertical bars with different letters indicate significant differences according to Duncan's multiple-range test ( $p < 0.05$ ) at a year of shelf life.

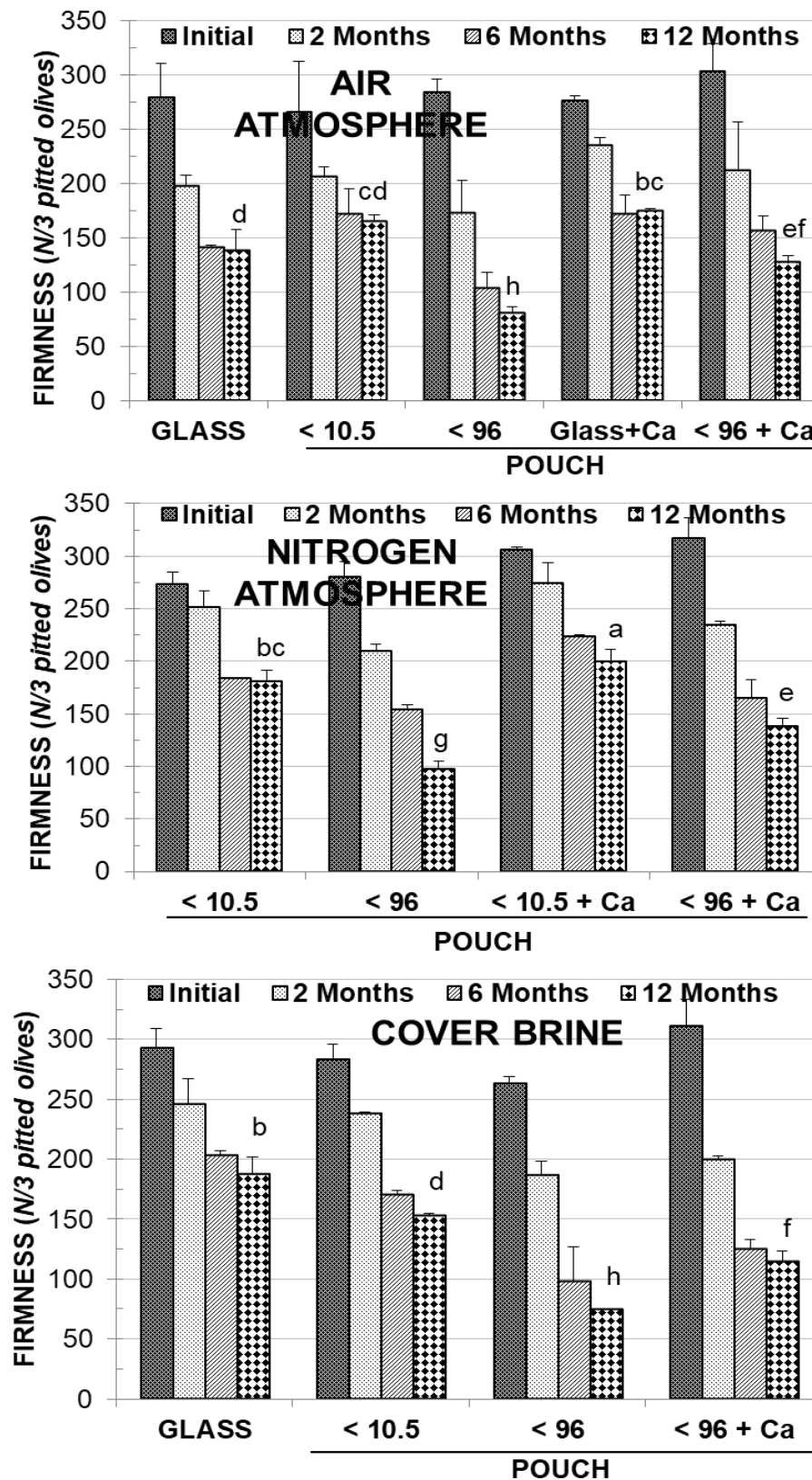


**Figure 2.** Evolution of the dissolved O<sub>2</sub> (mg/L) in the cover brine of jars and pouches containing acidified black ripe olives. Vertical bars with different letters indicate significant differences according to Duncan's multiple-range test ( $p < 0.05$ ) at a year of shelf life

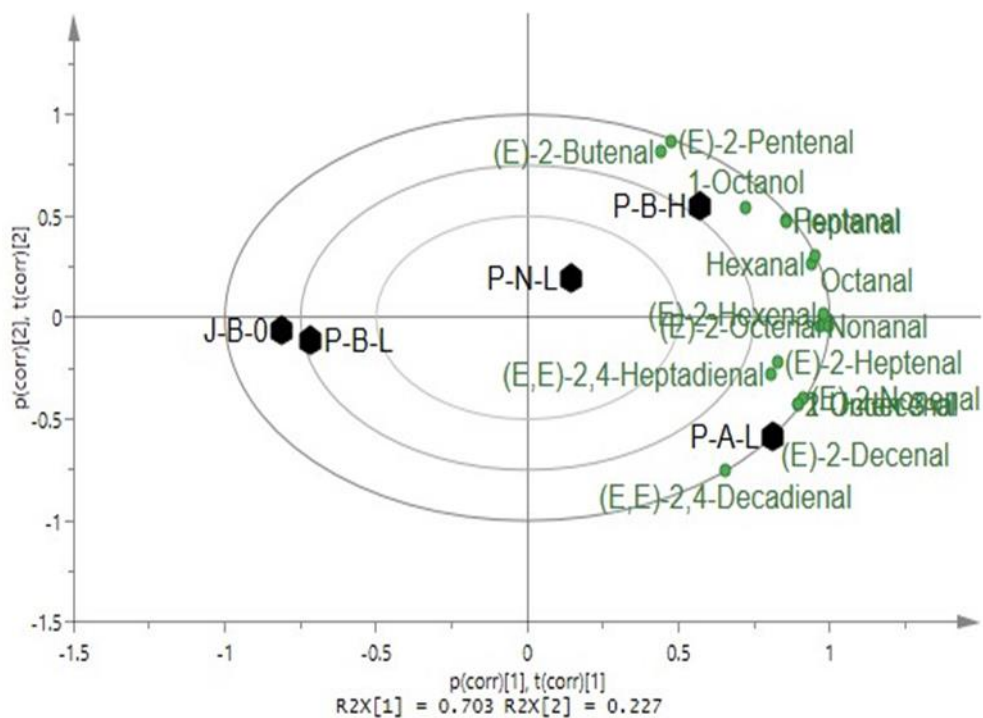




**Figure 3.** Superficial color ( $R_{700}$ ) of acidified black ripe olives packed in jars and pouches with air or nitrogen atmosphere and with cover brine. Vertical bars with different letters indicate significant differences according to Duncan's multiple-range test ( $p < 0.05$ ) at a year of shelf life.



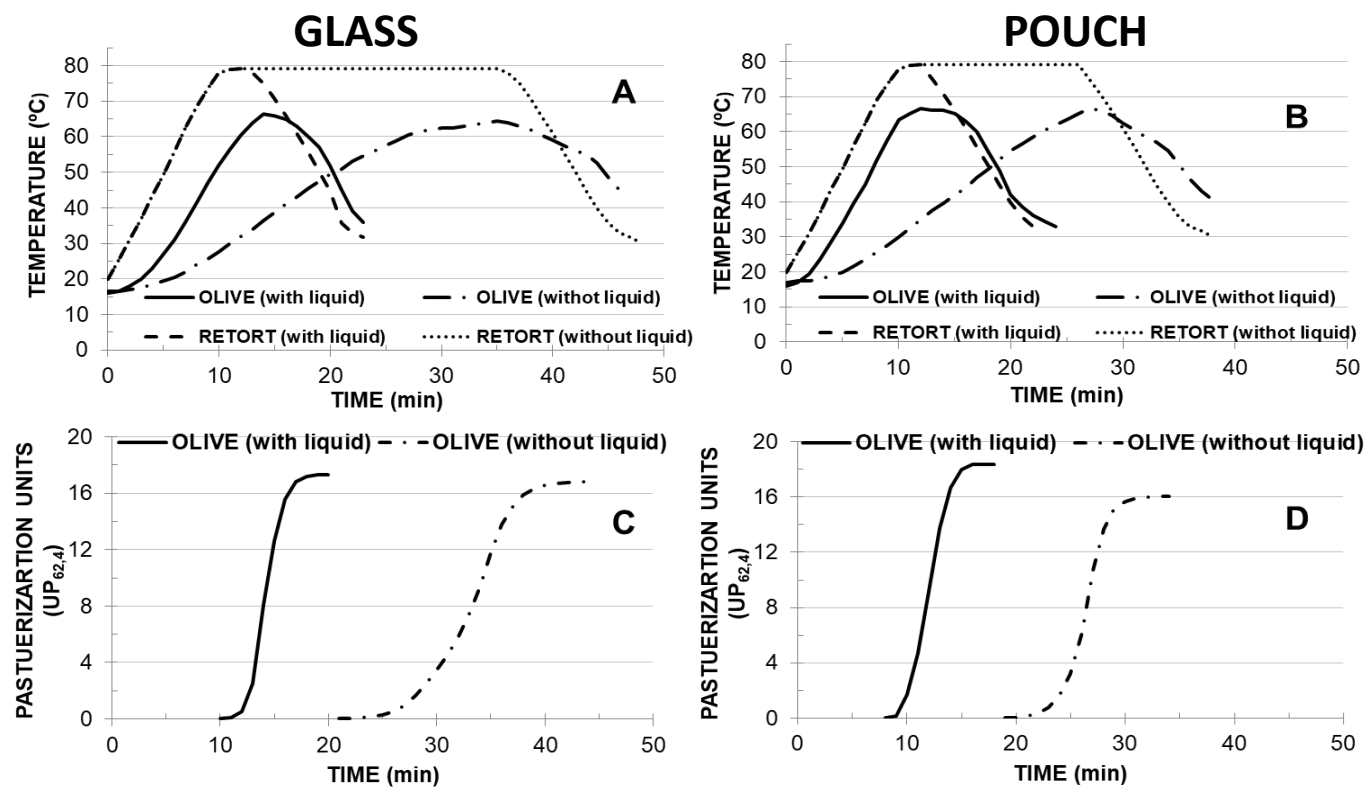
**Figure 4.** Firmness (N/3 pitted olives) of acidified ripe olives packed in jars and pouches with air or nitrogen atmosphere and with cover brine. Vertical bars with different letters indicate significant differences according to Duncan's multiple-range test ( $p < 0.05$ ) at a year of shelf life.



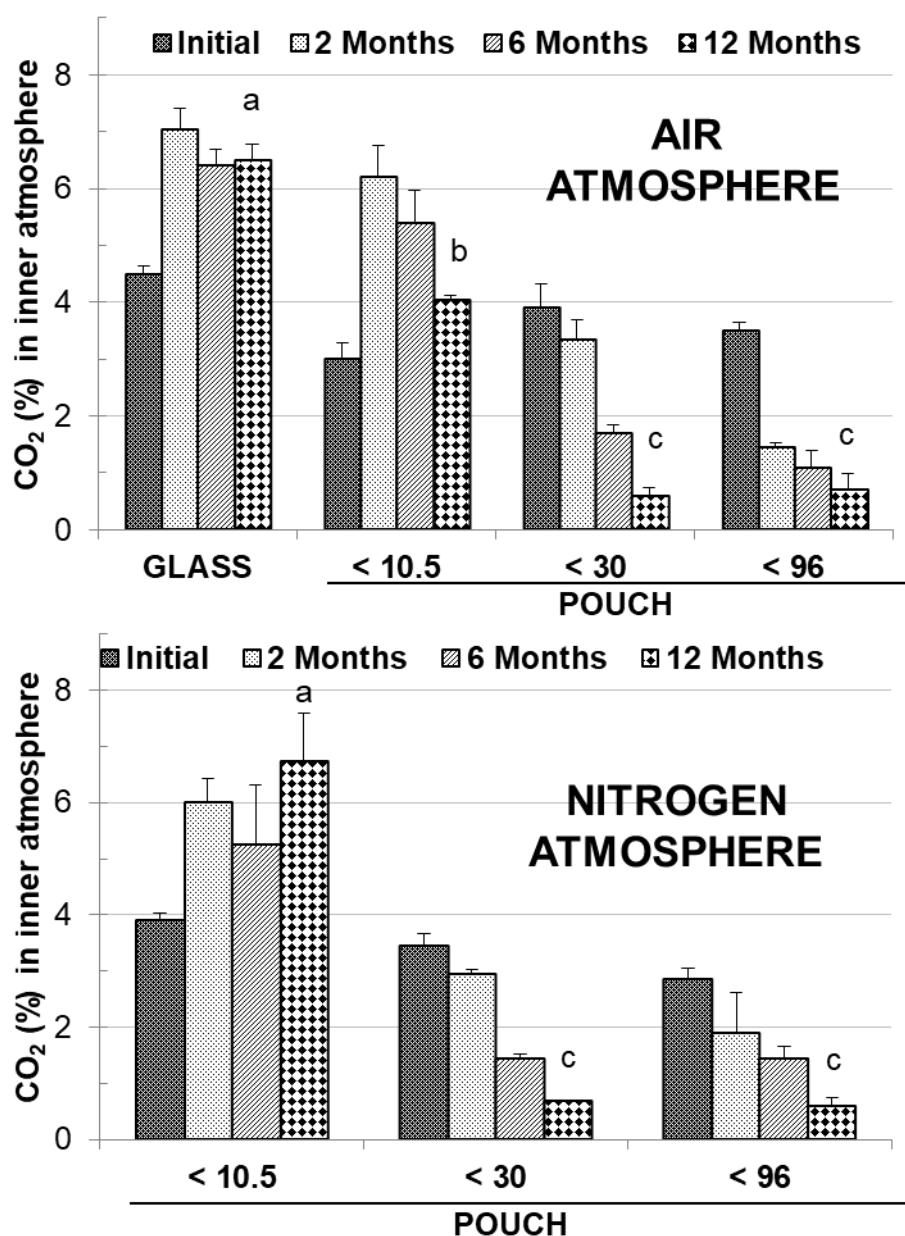
**Figure 5.** Principal component analysis (PCA) of volatile compounds derived from lipid oxidation in acidified ripe olives packed under different conditions. The acronyms of the packaging correspond to those in Table 1.

**Table S1.** Technical characteristics of the pouches used in the experiences with acidified black ripe olives

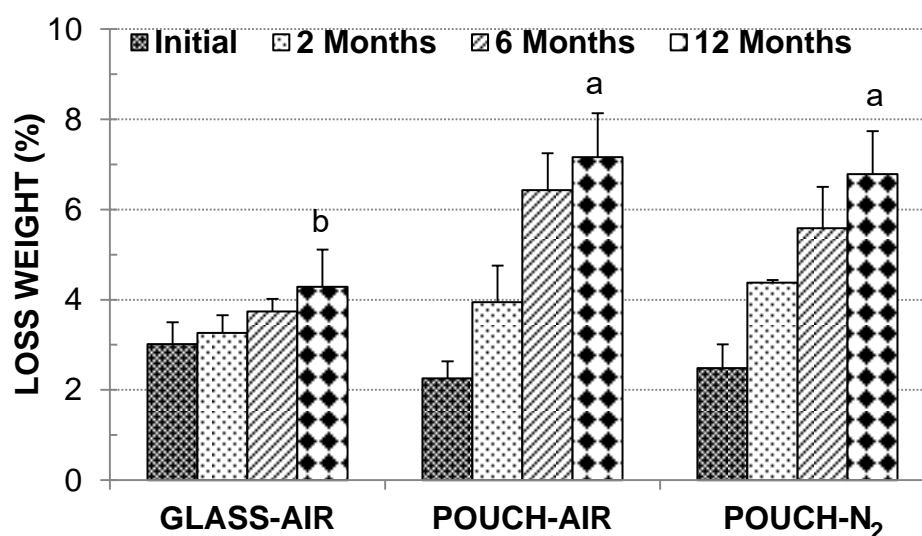
TECHNICAL DATA	UNIT	DESCRIPTION OF THE COMPLEX			METHOD
		High barrier polyester and polyethylene	Biaxially oriented polyamide and polyethylene	Polyester and white polyethylene	
		PET PVDC PAST+PE MD	OPA+PE MD	PET+PE B MD	
Oxygen permeability	cm <sup>3</sup> /m <sup>2</sup> /day	<10.5	<30	<96	ASTM D 3985
Thickness	µm	107 ± 10%	109 ± 10%	106 ± 10%	ASTM E 252
Density	g/cm <sup>3</sup>	1.00 ± 10%	0.96 ± 10%	1.00 ± 10%	ASTM D 1505
Unit weight	g/m <sup>2</sup>	107 ± 10%	104.5 ± 10%	105.7 ± 10%	ASTM D 252
Water vapor permeability	g/m <sup>2</sup> /day	<4.5	<8	<6	ASTM F 1249
Permeability to CO <sub>2</sub>	cm <sup>3</sup> /m <sup>2</sup> /day	<40	<118.5	<383	ASTM D 3985
Permeability to N <sub>2</sub>	cm <sup>3</sup> /m <sup>2</sup> /day	<2.5	<6	<19.5	ASTM D 3985
Departure sealing temperature	° C	120-130	120-130	120-130	SP GROUP 1 seg, 2 Bar



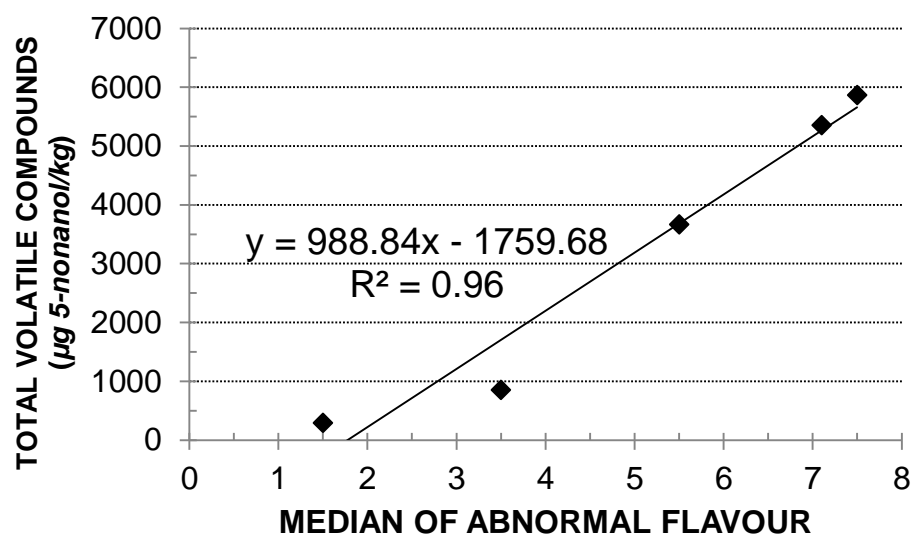
**Figure S1.** Evolution of the temperature in the retort and inside the glass jar (A) and plastic pouch (B) during pasteurization of acidified black ripe olives to reach an accumulated lethality of 15 UP<sub>62.4</sub> (glass jar, C; pouch, D). Olives were packed with and without cover brine.



**Figure S2.** Evolution of CO<sub>2</sub> (%) in the inner atmosphere of jars and pouches containing acidified black ripe olives. Vertical bars with different letters indicate significant differences according to Duncan's multiple-range test ( $p < 0.05$ ) at a year of shelf life.



**Figure S3.** Loss weight (%) of acidified black ripe olives packed in jars and pouches with oxygen permeability lower than 10, 30 and 96 cm<sup>3</sup>/m<sup>2</sup>/day under air or nitrogen atmosphere and cover brine. The analyses were carried out along a year. Vertical bars with different letters indicate significant differences according to Duncan's multiple-range test ( $p < 0.05$ ) at a year of shelf life.



**Figure S4.** Correlation between the total content of volatile compounds derived from lipid oxidation (aldehydes, ketones and alcohols) and the median of the perceptions of "abnormal flavor" obtained at the sensory evaluation.